AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

MINIREVIEW

The Complexities of *Nocardia* Taxonomy and Identification

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ABSTRACT *Nocardia* species are a complex group of organisms considered to belong to the aerobic actinomycetes. Of the validly described species, many have been implicated as the cause of serious human infections, especially in immunocompromised patients. The genus has a complicated taxonomic history; this is especially true for *Nocardia asteroides*, the type species of the genus and previously the most frequently reported nocardial taxon from human specimens. We provide background on the current taxonomy of *Nocardia*, with a focus on clinically relevant species, and discuss the currently available methods used to accurately identify isolates to the species, complex, or group level.

KEYWORDS aerobic actinomycetes, *Nocardia*, taxonomy

Prior to the molecular era, the taxonomy of *Nocardia* species and the identification of *Nocardia* isolates from patient specimens were relatively uncomplicated. In 1990, there were only a few known *Nocardia* species, and the routine identification of nocardial patient isolates in the clinical laboratory consisted of a small battery of phenotypic biochemical assimilation and utilization tests. With the advent of molecular methodologies, specifically, gene sequencing, a huge number of new *Nocardia* species have been identified. Even previously recognized species have undergone evaluation, reassessment, and inclusion into various groups and complexes. As new environmental species of *Nocardia* are identified, their significance in human infections, as well as the impact of *Nocardia* taxonomy, has been noted.

Currently in LPSN, the List of Prokaryotic names with Standing in the Literature (http://www.bacterio.net/index.html), there are 92 recognized species with valid names. Of these, 54 species have been shown to be clinically significant on the basis of at least one published report in the peer-reviewed literature (Table 1).

NOCARDIA TAXONOMIC HISTORY

The genus *Nocardia* has had a conflicted and confusing taxonomic history. In 1888, the veterinarian Edmond Nocard isolated a Gram-positive organism thought to be the causative agent of a case of bovine farcy (1). This strain was given the name *Nocardia farcinica* in 1889 by Trevisan (1), who defined the genus that included Nocard's *N. farcinica* isolate and five other species. Strains identified as *N. farcinica* were deposited in two separate culture collections (ATCC 3318 and NCTC 4524). In 1896, Eppinger isolated a branching filamentous organism, which he named *Cladothrix asteroides*, from a human brain abscess; this strain was subsequently renamed *Nocardia asteroides* (1). Additional species, including *Nocardia brasiliensis*, *Nocardia otitidiscaviarum*, and *Nocardia transvalensis*, and numerous other strains that formed fragmenting mycelia were subsequently included in the genus *Nocardia* (1). With the advent of chemotaxonomic methodologies, distinctions were made among isolates that were related to the original

Accepted manuscript posted online 8 November 2017

Citation Conville PS, Brown-Elliott BA, Smith T, Zelazny AM. 2018. The complexities of *Nocardia* taxonomy and identification. J Clin Microbiol 56:e01419-17. https://doi.org/10.1128/JCM.01419-17.

Editor Colleen Suzanne Kraft, Emory University

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TABLE 1 Current validly described *Nocardia* species of human clinical significance

Species	Year described	Commonly associated disease				
		Uncertain clinical significance[a]	Primary skin/ soft tissue	Pulmonary	Disseminated	Unknown source
N. abscessus	2000		+[b]	+	+	
N. africana	2001			+		
N. amamiensis	2007	+	+[b]	+		
N. amikacinitolerans	2013		+[b]	+		
N. anaemiae	2005	+				
N. aobensis	2005					+
N. araoensis	2004	+	+	+		
N. arthritidis	2004	+	+	+		
N. arizonensis	2015	+		+		
N. asiatica	2004			+		
N. asteroides	1891	+				
N. beijingensis	2001		+[b]	+	+	
N. blacklockiae	2008	+		+		
N. boironii	2016		+			
N. brasiliensis	1909		+[b]			
N. brevicatena	1961	+				
N. carnea	1891	+		+	+	
N. cerradoensis	2003	+		+	+	
N. concava	2005			+	+	
N. cyriacigeorgica	2001		+[b]	+	+	
N. donostiensis	2016	+		+		
N. elegans	2005			+	+	
N. exalbida	2006	+	+[b]			
N. farcinica	1889		+[b]	+	+	
N. harenae	2006	+	+			
N. higoensis	2004	+		+	+	
N. ignorata	2001	+	+			
N. inohanensis	2004	+	+			
N. jinanensis	2009	+	+			
N. kroppenstedtii	2014	+		+	+	
N. kruczakiae	2004		+[b]	+		
N. mexicana	2006		+	+	+	
N. mikamii	2010			+	+	
N. neocaledoniensis	2004	+	+			
N. niigatensis	2004		+			
N. ninae	2007	+		+		
N. niwae	2011			+		
N. nova	1983			+	+	
N. otitidiscaviarum	1924		+[b]	+	+	
N. paucivorans	2000				+	
N. pneumoniae	2004	+		+		
N. pseudobrasiliensis	1995			+	+	
N. puris	2003	+	+[b]		+	
N. shinanonensis	2016	+	+[b]			
N. sienata	2004	+		+		
N. takedensis	2005		+	+		
N. terpenica	2007	+		+		
N. testacea	2004	+		+		
N. thailandica	2005	+	+[b]	+		
N. transvalensis	1927		+[b]	+	+	
N. vermiculata	2005	+				
N. veterana	2001			+		
N. vulneris	2015		+			
N. wallacei	2006				+	
N. yamanashiensis	2004	+	+			

[a]Fewer than 4 cases published.
[b]Including ocular disease.

members of the genus *Nocardia* and some isolates that were sufficiently different to warrant inclusion in separate genera (such as the mycolic acid-containing genera *Gordonia*, *Rhodococcus*, and *Skermania* or other non-mycolic acid-containing genera). In 1954, *N. farcinica* (ATCC 3318/NCTC 4524) was designated the type strain of the genus *Nocardia* and species *farcinica*.

In 1962, Ruth Gordon and colleagues determined that the ATCC 3318 type strain of *N. farcinica* (as defined by Trevisan) could not be distinguished from isolates belonging to the species *N. asteroides* (as defined by Eppinger) with the use of the phenotypic tests available at the time. They concluded that *N. farcinica* and *N. asteroides* were two different names for the same species (1).

Further confusion in the taxonomic distinction of *N. farcinica* arose when the two strains of *N. farcinica* that were deposited in different culture collections were evaluated. Through the assessment of the organisms' mycolic acid contents, G+C values, and phage sensitivity studies, strain NCTC 4524 was determined to be more closely related to organisms in the genus *Mycobacterium*, while strain ATCC 3318 was related to the genus *Nocardia* (1). Because of the uncertainty related to the real isolate obtained by Nocard and to the apparent similarity of the type strains of *N. farcinica* and *N. asteroides* as noted by Gordon, an appeal was made to the Judicial Commission to have the type species of the genus *Nocardia* changed to *N. asteroides*, with strain ATCC 19247 designated the type strain of the genus. The *N. farcinica* strain ATCC 3318 was retained as the type strain of *N. farcinica*.

NOCARDIA ASTEROIDES

For many years, *N. asteroides* (with phenotypic characteristics similar to those of the type strain ATCC 19247) was implicated as the cause of numerous human infections and was considered to be the most commonly isolated *Nocardia* species (2). For identification purposes, clinical laboratories had access to only a limited battery of commercially available biochemicals, which mainly included tests that evaluated the abilities of isolates to hydrolyze or decompose adenine, casein, hypoxanthine, tyrosine, and xanthine. A retrospective analysis of the variety of *Nocardia* species isolated from clinical specimens shows that many of the currently recognized species are relatively inert in these hydrolysis media, giving negative reactions for all, as was typical for isolates of the type strain of *N. asteroides*; many human infections were therefore (probably incorrectly) attributed to *N. asteroides*. Indeed, as noted above, even the extended battery of biochemicals tested by Ruth Gordon indicated the biochemical similarity of *N. asteroides* and *N. farcinica*.

Goodfellow noted the complexity of the genus *N. asteroides* in his identification of the *N. asteroides* "cluster" (3). In 1988, Wallace et al. published an important observation regarding isolates identified biochemically as *N. asteroides*. Antimicrobial susceptibility testing of 78 consecutive *N. asteroides* isolates showed wide variability of antimicrobial susceptibility patterns among the isolates. These patterns grouped isolates into six different "drug pattern types." Because no phenotypic characteristics were available to biochemically differentiate the organisms that belonged to these drug pattern types (as all were relatively inert in the biochemicals used to identify them as noted above), the organisms biochemically similar to *N. asteroides* but with various antimicrobial susceptibility patterns came to be known as drug pattern types within the "*N. asteroides* complex." Interestingly, the type strain of *N. asteroides* fell into a "miscellaneous" group and showed a unique susceptibility pattern (4).

With the advent of molecular methodologies, including restriction fragment length analysis of both the heat shock protein (*hsp65*) gene and the 16S rRNA gene, clear molecular differences were seen among the members of the *N. asteroides* complex (5). Detailed molecular studies of the various drug pattern types followed the 1988 report. Sequence analysis of the 65-kDa *hsp65* gene and the 16S rRNA gene of isolates belonging to the various drug pattern types of the *N. asteroides* complex indicated that the gene sequences of the six drug pattern types were sufficiently different to ultimately warrant their division into six taxa. These included *N. abscessus* (drug pattern I), *Nocardia brevicatena/N. paucivorans* (drug pattern II), *Nocardia nova* complex (drug pattern III), *Nocardia transvalensis* complex (drug pattern IV), *N. farcinica* (drug pattern V), and *N. cyriacigeorgica* (drug pattern VI) (5). Because clear-cut phenotypic differences among these newly described species could not be identified, sequence analysis was determined to be the most accurate method for definitively identifying members of the

complex. Because these additional species can now be discriminated by molecular testing, the use of the term *N. asteroides* complex is no longer valid (6).

With this careful analysis of organisms phenotypically related to *N. asteroides*, it is clear that many of the organisms previously identified in patient specimens as *N. asteroides* were likely misidentified by today's standards, and most appear to be members of these differentiated species. Indeed, in Wallace's 1988 manuscript, the majority of "*N. asteroides*" isolates belonged to drug pattern type VI (35%). Isolates belonging to drug pattern type VI have subsequently been shown to belong to the species *N. cyriacigeorgica* (7) and may likely be the species responsible for the majority of human infections reported prior to the availability of molecular tests. Organisms genetically identical to the type strain of *N. asteroides* are rare isolated from clinical samples, and this species is no longer considered to be the most commonly isolated *Nocardia* species from human specimens (5, 6).

IDENTIFICATION OF *NOCARDIA* SPECIES

The various *Nocardia* species have different geographic prevalences, pathogenic traits, and antimicrobial susceptibility patterns. Therefore, the identification of *Nocardia* isolates to the species level is important to provide the appropriate level of patient care. This level of identification may be difficult for many clinical labs that do not have the financial or technical capabilities shown to be useful for the identification of *Nocardia* species.

Because of the large number of recognized *Nocardia* species, biochemical methods are clearly insufficient to accurately discriminate among the clinically relevant species. There are relatively few biochemical tests available compared to the number of possible species, and the interpretation of results of biochemical testing requires extended incubation and specific expertise. In addition, while the description of many newly recognized species includes some biochemical data, the types of biochemical tests reported as typical for the new species are not consistent from species to species, making a comparative identification impossible. In the light of currently available methodologies that can provide accurate and rapid results (gene sequencing and matrix-assisted laser desorption ionization–time of flight mass spectrometry [MALDI-TOF MS]), the use of biochemical testing is neither accurate nor timely.

Molecular methodologies, specifically, gene sequencing, have become the most accurate for definitively identifying *Nocardia* to the species level. Numerous genes, including 16S rRNA, *secA1*, *hsp65*, *gyrA*, and *rpoB*, have been evaluated for their ability to adequately discriminate among the various *Nocardia* species. By far, most of the information available in public databases is for the 16S rRNA gene. All *Nocardia* species include a variable region near the 5′ terminus of the gene (5), which allows for the differentiation of the majority of the currently recognized *Nocardia* species with a partial (500 bp) 16S rRNA gene sequence. Extended sequencing of the entire gene may be required for the recognition of a new species or for the discrimination of closely related species that have identical, or nearly identical, sequences within the first 500 bp of the gene, such as in *N. abscessus/N. asiatica/N. arthritidis*, *N. elegans/N. veterana*, *N. higoensis/N. shimofusensis*, *N. farcinica/N. kroppenstedtii*, and *N. brasiliensis/N. vulneris*.

A major difficulty with the use of the 16S rRNA gene for the identification of *Nocardia* species is the high level of sequence similarity among species that have been shown to be distinct by DNA-DNA hybridization studies. In some instances, only a few base differences separate closely related species, making an unambiguous identification of some isolates difficult. For example, the species pairs *N. brevicatena/N. paucivorans* and *N. kruczakiae/N. veterana* show 99.5% (1,352 bp) and 99.8% (1,379 bp) 16S rRNA gene sequence similarities, respectively (5). The CLSI document MM18-A recommends a sequence similarity equal to or greater than 99.6% (with greater than 0.4% separation between different species) for the identification of *Nocardia* isolates to the species level (8). MM18-A also provides recommendations for percentage sequence similarity of 99.0 to 99.5% for identification to the genus level. Figure 1 demonstrates

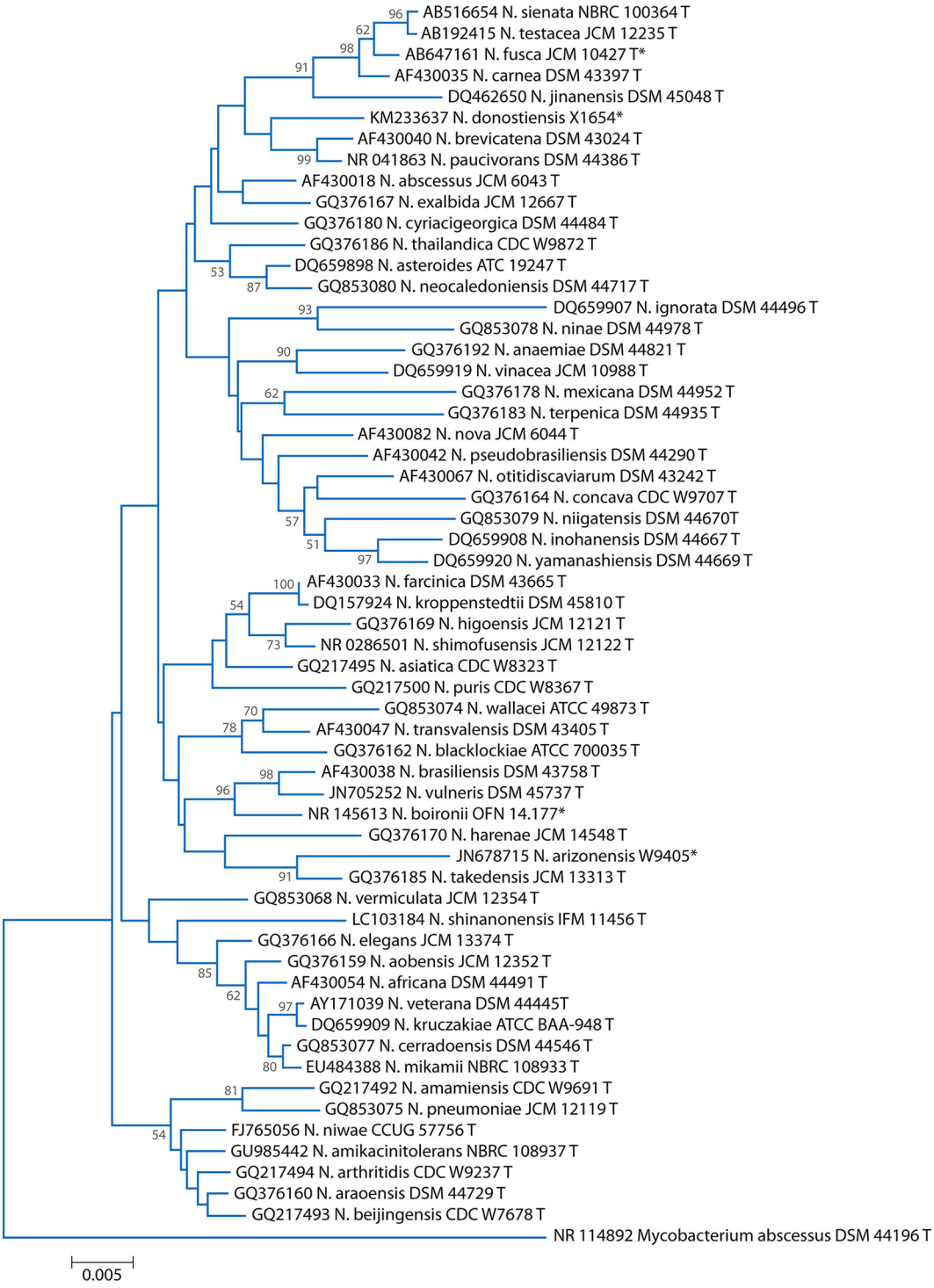

FIG 1 Alignment of 1,427 bases of the 16S rRNA gene of 59 *Nocardia* species obtained using the neighbor-joining (25) method of MEGA6 (26) software. The tree is drawn to scale. Bootstrap replicate percentages greater than 50% (1,000 replicates) are shown next to the branches (27). The evolutionary distances were computed using the maximum composite likelihood method (28) and are in the units of number of base substitutions per site. T, type strain; *, species not yet validly published.

the close alignment of *Nocardia* species as determined by the alignment of 1,427 bases of the 16S rRNA gene.

When used for the identification of *Nocardia* isolates, the sequence chromatograms should be carefully evaluated for evidence of multiple copies of the 16S rRNA gene with dissimilar nucleotide sequences, which may complicate definitive identification. The

presence of multiple copies is demonstrated by overlapping peaks at specific positions in Sanger sequencing chromatograms, especially in the variable region of the gene (see above). These multiple peaks are not resolved with repeat extraction and Sanger sequencing; cloning and sequence analysis of the resulting clones have been used for definitive identification (9).

Sequence analysis of a 468-bp region of the housekeeping gene *secA1* has been shown to be particularly useful for the accurate discrimination of *Nocardia* species (10). In addition, an alignment of the deduced amino acid sequence (156 amino acid residues) provides additional information regarding species identity, as the analysis of the deduced amino acid resolves some within-species base differences that could complicate analysis of gene sequences alone. The intraspecies microheterogeneities seen with the analysis of the *secA1* gene sequence may be useful as an epidemiologic tool, especially among *N. nova*, *N. cyriacigeorgica*, *N. farcinica*, and *N. veterana* isolates (11). The *secA1* gene sequences of many *Nocardia* spp. are included in GenBank; however, *secA1* sequences of more recently described species may not yet be available.

An evaluation of the phylogenetic trees of *Nocardia* species shows some distinct species that are closely related based on the similarity of their gene sequences. One such grouping that is commonly reported is the *N. abscessus* complex, which includes members of *N. abscessus*, *N. arthritidis*, *N. asiatica*, and *N. beijingensis*. While members of this complex may be discriminated on the basis of the full 16S rRNA gene sequence, an analysis of the first 500 bp may not provide a sufficient basis for species discrimination. Interestingly, even though these isolates cluster together on the basis of their gene sequences, there is no single antimicrobial susceptibility pattern that is common to all species in the complex (our unpublished data).

Decreasing costs and turnaround times are making whole-genome sequencing a viable approach for clinical laboratories; the increasing availability of this technology and its careful integration into identification algorithms may facilitate accurate discrimination among closely related species and timely recognition of new species.

MALDI-TOF MS

Recently, MALDI-TOF MS has been shown to provide accurate identification of *Nocardia* species when an augmented *Nocardia* library is employed. However, while some species are easily identified (i.e., *N. brasiliensis*), for others, the identification has only been shown to extend to the complex level (*N. abscessus* complex, *N. brevicatena-N. paucivorans* complex, *N. nova* complex, and *N. transvalensis* complex) (12–14). The identification of uncommon species remains a challenge. Variation in the spectral profiles exist for some *Nocardia* species (i.e., *N. cyriacigeorgica*), and it is unclear if these variations represent taxonomic heterogeneity (15, 16). When developing custom user-built *Nocardia* identification databases to supplement commercial databases, laboratories should include spectra from well-characterized organisms, including the type strain and other reference strains, with the goal of successfully identifying the most common species/complexes for that institution. Users should also be aware of the extraction method used to develop the database, as superior performance has been noted when the same extraction method is used for both database development and the testing of unknown isolates (17).

Several studies have demonstrated the limitations of commercial databases for providing accurate identifications with variable (between 0% to 47.3%) agreement to sequencing methods (12, 15, 16). With the use of in-house libraries developed with custom spectra, identification agreement improved to up to 95% agreement (12, 14, 16).

A comparison among studies using the different platforms is not straightforward due to major differences in databases (commercial and/or customized), strains used to challenge them, criteria for acceptable identification, and a disproportionate number of studies with each platform.

For the first commercially available MALDI-TOF MS system, a large number of studies show a satisfactory-to-very good performance using a customized database along with the commercial one. Overall, successful identification was achieved with common species, including *N. brasiliensis*, *N. cyriacigeorgica*, *N. farcinica*, *N. nova/N. nova* com-

plex, and *N. otitidiscaviarum*. Variable results were obtained for *N. transvalensis/transvalensis* complex, *N. pseudobrasiliensis*, *N. abscessus/abscessus* complex, and *N. beijingensis*. As expected, uncommon species with limited or no representation in the databases were the most challenging (i.e., *N. aobensis*, *N. testacea*, and *Nocardia* spp.) (12, 13). A total of 46 *Nocardia* species strains (belonging to 12 species) were used in the evaluation of a second commercial system, yielding 91% correct species-level identification (18). Two recent publications by Girard et al. (19, 20) discuss the development and validation of a *Nocardia* MALDI-TOF MS database to be released in an updated database for a third commercial system. A challenge set of 164 strains yielded 95% correct identification of claimed species. The platform successfully identified the common species *N. cyriacigeorgica*, *N. farcinica*, *N. nova/N. nova* complex, and *N. otitidiscaviarum* and also the less common species *N. abscessus* and *N. beijingensis*. However, while most (6/7) *N. brasiliensis* isolates tested were correctly identified, one strain was misidentified as *N. farcinica* (reason unclear). In addition, *N. transvalensis* could not be tested due to the lack of strains. Additional studies are needed to further evaluate the performance of the commercial systems for which only a limited number of studies with *Nocardia* spp. is available.

MULTILOCUS SEQUENCE ANALYSIS

While 16S rRNA gene sequencing is generally considered the gold standard for bacterial identification, it fails to discriminate among some species of *Nocardia*. Multilocus sequence analysis (MLSA) using concatenated sequences of 4 to 5 housekeeping genes (16S rRNA, *gyrB*, *secA*, *hsp65*, and/or *rpo*B) has been increasingly used to provide higher accuracy and discriminatory power in the molecular identification of *Nocardia* spp., to gain information on taxonomic delineations, and more recently, to help reconcile discrepancies between single target sequence-based and MALDI-TOF MS-based identification results (15, 16, 21).

MLSA data revealed striking differences in intraspecies strain diversity with high-diversity species such as *N. cyriacigeorgica*, with strains clustering in 3 distinct subgroups compared with a much lower diversity among *N. farcinica* strains.

While MLSA does provide higher accuracy in the identification of isolates, it has limitations. (i) It does not resolve the identification of some isolates in some challenging species, groups of species, or complexes (i.e., *N. abscessus/N. arthritidis*-like species within the *N. transvalensis* complex). (ii) It is cumbersome for many clinical laboratories to sequence 4 or 5 genes to identify clinical isolates of *Nocardia* spp. Therefore, abbreviated MLSA schemes with 3 (or even 2) targets have been proposed (i.e., 16S rRNA, *gyrB*, and *secA* [21]), which provide species (or complex) assignment for the majority of isolates or can raise suspicion on the occurrence of a novel species.

EXTENT OF IDENTIFICATION AND REPORTING

Depending on the method used for the characterization of *Nocardia* species, isolates may be identified to a "complex" or "group" level. These identification methods may group species on the basis of a specific characteristic that defines the complex. *Nocardia* spp. have been classified into complexes on the basis of various phenotypic or genotypic attributes, including their antimicrobial susceptibility patterns, by the similarity of their gene sequences, and by their proteomic profiles as determined by MALDI-TOF MS analysis.

A number of species are grouped into complexes on the basis of their predicted antimicrobial susceptibility patterns. Based on susceptibility test results, *Nocardia* organisms can be grouped into the following complexes: the *N. nova* complex (*N. nova*, *N. elegans*, *N. veterana*, *N. kruczakiae*, and *N. africana*), *N. transvalensis* complex (*N. blacklockiae* and *N. wallacei*, unnamed *Nocardia* sp.), and the *N. brevicatena/N. paucivorans* complex (Table 2).

Isolates may be clustered into a single species complex on the basis of the variation of gene sequences observed for isolates belonging to a certain species.

TABLE 2 Commonly designated complexes of *Nocardia* species

Complex	Basis for complexing	Species included in the complex
N. abscessus complex	MALDI-TOF MS profile	*N. abscessus*, *N. arthritidis*, *N. asiatica*, *N. beijingensis*, *N. pneumoniae*
	Gene sequence (500 bp of 16S rRNA)	*N. abscessus*, *N. arthritidis*, *N. asiatica*, *N. beijingensis*
N. nova complex	Gene sequences (16S rRNA and/or *secA1*)	*N. africana*, *N. aobensis*, *N. cerradoensis*, *N. elegans*, *N. kruczakiae*, *N. mikamii*, *N. nova*, *N. vermiculata*, *N. veterana*
	Antibiotic susceptibility pattern	*N. africana*, *N. elegans*, *N. kruczakiae*, *N. nova*, *N. veterana*
	MALDI-TOF MS profile	*N. africana*, *N. aobensis*, *N. elegans*, *N. kruczakiae*, *N. nova*, *N. veterana*
N. transvalensis complex	Antibiotic susceptibility pattern	*N. blacklockiae*, *N. transvalensis*, *N. wallacei*
	Gene sequences (16S rRNA, *secA1*)	
	MALDI-TOF MS profile	
N. brevicatena/*N. paucivorans* complex	Antibiotic susceptibility pattern	*N. brevicatena*, *N. paucivorans*
	Gene sequences (16S rRNA, *secA1*)	
	MALDI-TOF MS profile	
N. otitidiscaviarum complex	Gene sequences (16S rRNA, *hsp65*)	Various strains of *N. otitidiscaviarum*
N. cyriacigeorgica complex	16S rRNA gene sequence	Various strains of *N. cyriacigeorgica*
N. farcinica complex	Gene sequences (16S rRNA, *secA1*)	*N. farcinica*, *N. kroppenstedtii*

Patel et al. noted variation in the sequences of the 65-kDa *hsp65* and 16S rRNA genes of a collection of *N. otitidiscaviarum* isolates and determined that the species may actually include a complex of isolates with closely related species (22). Schlaberg et al. noted the heterogeneity of both the *hsp65* and 16S rRNA genes of isolates belonging to the species *N. cyriacigeorgica* (23). Various distance matrix methodologies have been used to analyze the relatedness of the 16S rRNA genes of isolates belonging to a particular species and have shown that while differences exist in the gene sequences of those isolates, most isolates of a species cluster around a "centroid" sequence (24).

For laboratories with limited resources and/or access to newer technologies (gene sequencing and MALDI-TOF MS), identification to the species or even complex level may not be possible. For such laboratories, we suggest that presumptive identification at least to the *Nocardia* genus level is desirable and can be achieved by careful evaluation of phenotypic characteristics (modified acid-fast positive, branching rods, and presence of aerial hyphae) of the isolate (9). If treatment is indicated based on the specimen from which the isolate was recovered and on clinical indications, antimicrobial susceptibility testing should be performed in-house or by a reference laboratory.

Due to the ubiquitous nature of *Nocardia*, a report of the presence of this organism in a clinical specimen can be confusing or even misleading to patient care professionals. Any report of a *Nocardia* species should be followed by direct communication between the clinical and laboratory staff to establish the significance of the isolate and to determine the need for additional testing, including antimicrobial susceptibility tests (9).

Laboratory reports that include the identification of *Nocardia* isolates at a complex or group level should include a clearly stated basis for the complex determination; the reports should also indicate the organisms that are considered to be included in the complex or group and if members of the complex are known to possess similar antimicrobial susceptibility profiles.

PROPOSED ALGORITHM FOR LABORATORY IDENTIFICATION OF *NOCARDIA* SPECIES

While MALDI-TOF MS can provide an accurate species- or complex-level identification of the most commonly isolated *Nocardia* species, the use of gene sequencing may be required for the definitive identification of members of a complex or for unusual species. The flowchart shown in Fig. 2 illustrates an identification pathway for different

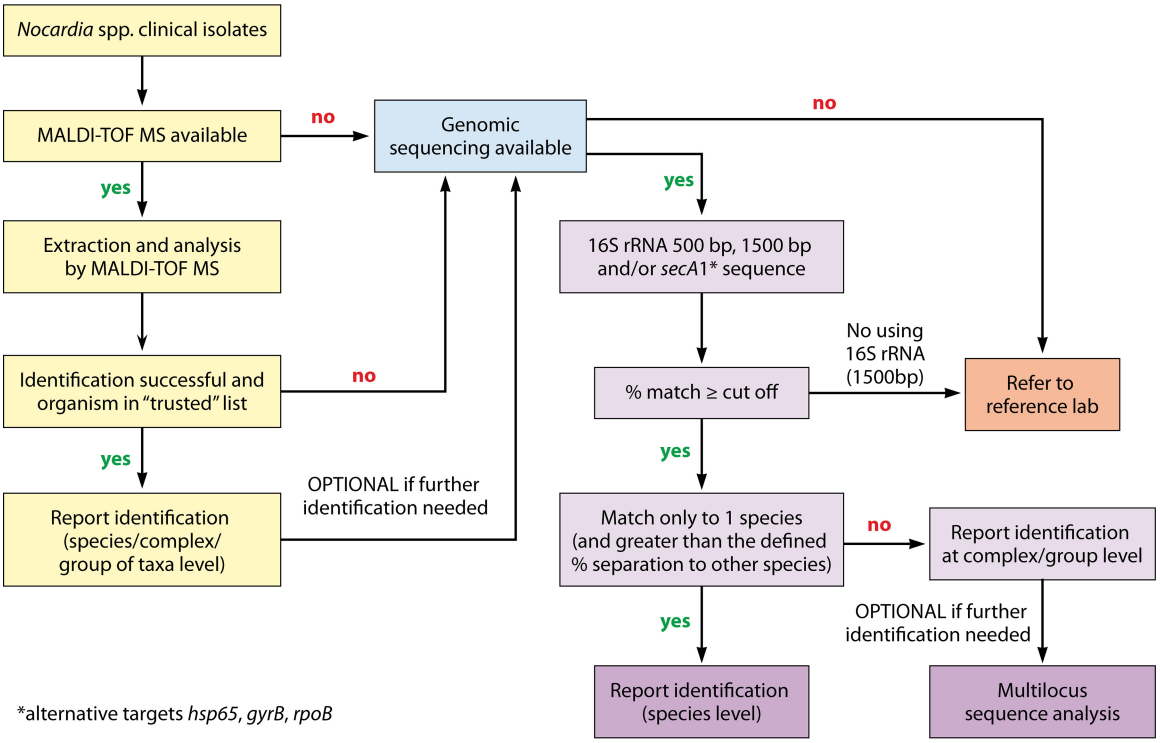

FIG 2 Identification methodology pathway that can be employed to achieve accurate identification of *Nocardia* clinical isolates.

laboratory capabilities (i.e., with or without MALDI-TOF MS), utilizing gene sequencing to achieve accurate isolate identification.

SUMMARY AND PROPOSED ALGORITHM FOR LABORATORY IDENTIFICATION OF *NOCARDIA* SPECIES

The goal of this review was to present a clinically focused taxonomic update of the genus *Nocardia*, highlighting the present status of commonly isolated species and current microbial identification methods. The chart proposes the use of the increasingly used MALDI-TOF MS systems, validated for the most common species of *Nocardia*, as the first identification method in order to minimize the number of strains requiring genomic sequencing or referral to a reference center for identification. This chart relies on the availability of MALDI-TOF MS or genomic sequencing for the accurate identification of *Nocardia* isolates to the complex or species level.

ACKNOWLEDGMENT

The views expressed here are those of the authors and should not be construed as those of the U.S. Department of Health and Human Services.

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